

RESPONSE OF *MDR1* AND *PDR1* ATP-BINDING CASSETTE-TYPE TRANSPORTER GENES TO BIOTIC SIGNALS IN WHEAT FLAG LEAF TIPS

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Abstract: ATP-binding cassette-type (ABC) transporters are highly implicated in detoxification processes but not restricted to detoxification processes. Several ABC transporters including wheat Lr34 were shown to function in plant defense responses and secretion of plant antimicrobial compounds. Members of multidrug resistance (MDR) proteins and pleiotropic drug resistance (PDR) proteins were studied in wheat. MDR1 and PDR1 expression was relatively stable in all the developmental stages but responded differentially to salicylic acid and fumonisin B1. *In silico* analysis indicated that both MDR1 and PDR1 had expression levels in all analyzed parts of wheat.

Keywords: ABC-transporter, biotic stress, disease resistance, protein kinase, wheat Lr34.

Introduction

Plants must continuously defend themselves against attack from pathogens. Because their immobility precludes escape, plants possess both a preformed and an inducible defense capacity. Toxin detoxification represents one of the most effective defense mechanisms and one of the most significant findings was from the study wheat stripe rust resistance gene *Lr34* [KRATTINGER & al. 2011]. *Lr34* has been associated with both wheat leaf rust and stripe rust [SINGH, 1992]. It is a widely-studied benchmark for multiple fungal pathogen resistance and is controlled by a single ATP-binding cassette (ABC) transporter gene [KRATTINGER & al. 2011].

Confirmation of the hypothesized ABC transporter motif is a significant step in understanding disease resistance in wheat, as disease resistance protein in *Arabidopsis* homologs have been documented as having ABC transporter motifs [KANG & al. 2011]. First identified as transporter proteins located in eucaryotic cell membranes, ABC proteins have been found to be ubiquitous and their role in cellular metabolism to be broader than transporting molecules across the cell membrane. The common unifying characteristic of ABC proteins is a unique amino acid signature sequence. In addition, the ABC proteins are a constitution of subunits forming hetero- or homodimer. The subunits consist of a transmembrane domain (TMD) and nucleotide-binding domain (NDB). The number and general combination of subunits of ABC are varied but generally fall into nine subfamilies. ABC proteins in the subfamilies Pleiotropic Drug Resistant (PDR), Multidrug Resistance (MDR) and Multidrug Resistance-associated Protein (MRP), have been documented as having a role in managing biotic and abiotic stresses in other organisms [SHANG & al. 2011; SASAKI & al. 2002; THEODOULOU & al. 2003].

LR34 is predominantly expressed in adult foliar tissues, particularly of the flag leaf, and the highest transcript levels were found in the leaf tip, corresponding to the tissues

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that exhibit the phenotypic difference between the tolerant and susceptible wheat lines [KRATTINGER & al. 2009]. Wheat varieties with functional *LR34* alleles can be distinguished phenotypically by the development of leaf tip necrosis in adult flag leaves [KRATTINGER & al. 2009; KANG & al. 2011]. Despite its resistance-conferring properties, *LR34* is not responsive to pathogen inoculation, suggesting that it has constitutive rather than induced functions [KANG & al. 2011]. Here, we report our study on differential expression of different ABC transporters in flag leaves of Fielder cultivar, which does not possess expression resistance genes when challenged with stripe rust [RANDHAWA & al. 2012]. We also studied the effect of transient expression of a wheat mitogen-activated protein kinase on the expression of MDR and PDR. Our data suggest that the tested ABC proteins could be induced in a stripe rust-susceptible cultivar.

Material and methods

Plant growth

Triticum aestivum seeds of the Fielder variety were sterilized in a solution of 70% ethanol for 2 min, then transferred to a bleach solution of 25 ml of bleach, 25 ml of distilled water and 10 µl of triton extract. The seeds were then rinsed 10 times in distilled water. After drying 5-6 seeds were potted in autoclaved Pro-mix BX soil fertilized with 7-9 granular of slow release NPK fertilizer (14:14:14). The seeds were then placed in a growth chamber set for 16 hr at 22 °C in the light and 8 hr at 18 °C in the dark. The seed were watered every second day.

Treatment with fumonisin B1 and salicylic acid

Wheat seeds were left to grow for a period of three weeks. The leaves were harvested and cut into segments of 1.5-2 cm and were treated 5µM fumonisin B1 (FB1, Sigma-Aldrich) and 100 µM salicylic acid (SA, Sigma-Aldrich) or ddH₂O as a control. The leaves were then placed in a vacuum infiltration chamber for a period of 30 minutes. The treated leaf segments are then placed upwards on top of a filter paper in a Petri dish. At intervals of 0 hr, 6 hr, 24 hr and 48 hr the leaf segments were collected and placed in Falcon tubes and snap frozen in liquid nitrogen. The materials were then stored in a -80 °C. Protein kinase inhibitors staurosporine and SB202190 were from Sigma-Aldrich Canada.

RNA extraction

Total RNA was extracted from wheat leaf tissues (100 mg) using TRIzol Reagent Kit (Invitrogen) according to manufacturer's protocol. After TRIzol extraction, deoxyribonuclease I kit (Invitrogen) was used to eliminate genomic DNA contamination in the sample, and the cloned AMV First-Strand cDNA Synthesis Kit (Invitrogen) was used for cDNA synthesis according to the manufacturer's protocol. RT-PCR was carried out under the following conditions: 94 °C for 1 min; 1 min at 94 °C, 1 min at 61 °C, and 1 min at 72 °C for 25 cycles; and then 10 min at 72 °C.

RT-PCR

PCR primers for *Triticum aestivum* PDR-type ABC transporter (*PDR1*) and *Triticum aestivum* MDR-type ABC transporter (*MDR1*) were designed using NCBI's Primer-BLAST tool. RT-PCR was performed for *PDR1* (FJ185035.1) and *MDR1* (AB055077.1). Wheat *PDR1* primers were 5'-GACCGTAAGAGACGCTCG-3' (forward) and 5'-GCAGGAGG

GAGATCATCACG-3' (reverse). Wheat *MDR1* primers were 5'-GCAGAGAAAAGAGTGG TTACAAC-3' (forward) and 5'-AGGAATGTGCAAGGTAAAGTCAC-3' (reverse). Wheat actin was used to standardize the volume of cDNA loading per treatment. The following conditions were used for RT-PCR: 94 °C for 1 min, 60 °C for 1 min, and 72 °C for 30 seconds for 28 cycles, and then 10 min at 72 °C.

Gene expression analysis with Geneinvestigator

GenBank accession numbers AB055077.1 and FJ185035.1 were used for *Triticum aestivum* *PDR1* and *MDR1*, respectively. From the Geneinvestigator databases, target sample probes, either from *T. aestivum* or a related cereal organism, were identified. The target probes were put through Condition Search in Geneinvestigator to identify sample experiments and expression levels in the anatomy.

Results and discussions

Effect of fumonasin B1 and salicylic acid on *MDR1* and *PDR1* expression

As shown in Figure 1, FB1 and SA up-regulated both genes relative to the treatment with water, the control. The expression patterns between the FB1 and SA treatment across both genes are generally similar (Figure 1). Compared to the control *MDR1* expression seemed to be sustained through to the 6 hrs inoculation period, thereafter the expression level decreased for both SA and FB1 treatment. FB1 and SA did not affect expression at 0 hrs across all treatments as indicated by similar band strengths as the control. *MDR1* expression was slightly more consistent when treated with FB1 over SA treatments. *PDR1* expression was observed when treated with either FB1 or SA, no expression was observed in the control at any time interval.



Figure 1. Effect of fumonasin B1 and salicylic acid on *MDR1* and *PDR1* expression. The effect was analysed by RT-PCR. Actin was used as internal standard. This experiment was performed three times with similar results.

Effect of protein kinase inhibitors on FB1- or SA-induced expression of *MDR1* and *PDR1*

To study the downstream signaling pathways FB1- or SA-induced *MDR1* and *PDR1* gene activation was examined in the presence of staurosporine, a broad spectrum protein kinase inhibitor, and SB 202190, an inhibitor specific to the p38 class MAPKs. Staurosporine reduced the FB1- or SA-induced expression of *MDR1* and *PDR1*, whereas inhibitors of p38 class MAPK had no effect (Figure 2).

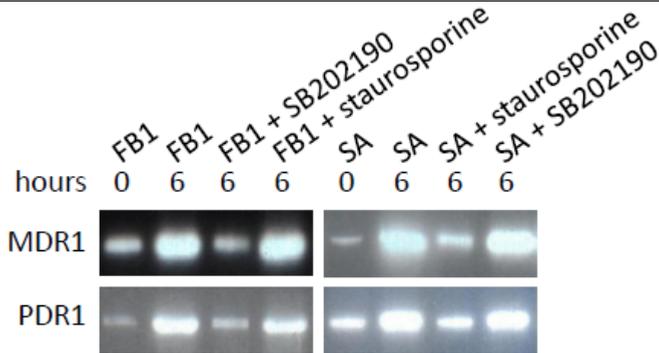


Figure 2. Effect of protein kinase inhibitors on FB1- or SA-induced expression of *MDR1* and *PDR1*. The effect was analysed by RT-PCR. Kinase inhibitors (kinase inhibitors at the concentration of 1 μ M for staurosporine and 350 nM for SB 202190) were included in the treatment. Actin was used as an internal standard. This experiment was performed three times with similar results.

Expression analysis of *MDR1* and *PDR1* with Geninvestigator

In silico analysis indicated that both *MDR1* and *PDR1* had expression levels in all analyzed parts of wheat. The expression strength is equitably distributed across all parts of the plant as no part of the plant was statistically different from the other (Figure 3). There is no significant difference in expression in the various plant organs.

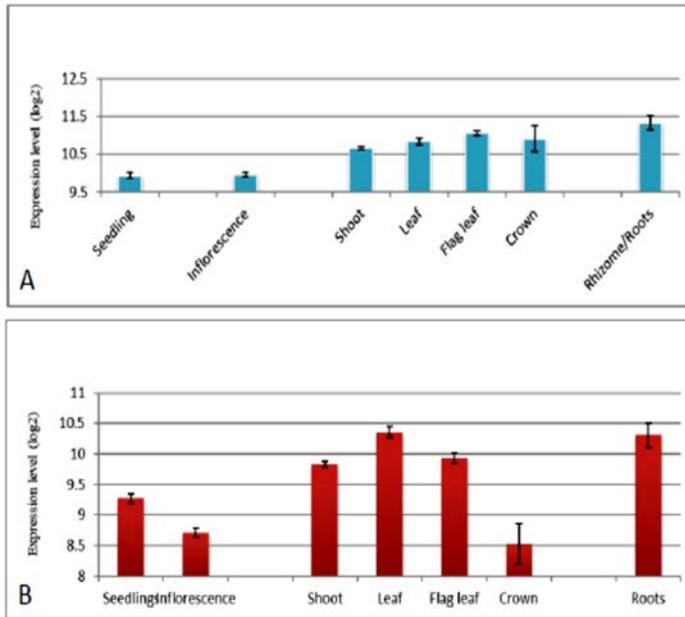


Figure 3. Expression levels across anatomical parts of the plant. A. *PDR1*, B. *MDR1*.

Discussion

The study of ABC transporters in plant disease resistance has been much enhanced with the confirmation of the presence of an ABC transporter motif within the Lr34/Yr18/Pm38 coding region [KRATTINGER & al. 2009]. Later on, Lr34 was also shown to function as a transporter of the ABCG subfamily [KRATTINGER & al. 2011]. A family of ABCG transporter genes in wheat cultivars was shown to be enhanced when wheat plants were challenged by a deoxynivalenol-producing *Fusarium graminearum* strain [MUHOVSKI & al. 2014]. Some ABC transporters such as Lr34 were shown to function in secretion of plant antimicrobial compounds [KRATTINGER & al. 2011; HWANG & al. 2016]. Here, members of multidrug resistance (MDR) proteins and pleiotropic drug resistance (PDR) genes in wheat were studied.

At time 0 hr *PDR1* levels are very low or non-existent. Leaf samples treated with H₂O showed no expression at any of the treatment time intervals. When treated with FB1 and SA *PDR1* expression was enhanced. The results appear to mirror previous research where members of the PDR family of ABC were expressed and up-regulated in the presence of biotic and abiotic stress [KANG & al. 2011]. PDR is localized on the plasma membrane [KANG & al. 2011], thus ideal for early detection of any pathogen. *MDR1* expression pattern was similar whereby the bands at all time intervals were more pronounced when treated with SA and FB1 in comparison to the control (Figure 1). In the control, bands of *MDR1* was observed, though faint, indicating there is a background level of MDR1 while the cell is in homeostasis. SA is known to be involved in plant defense [ZHANG & LI, 2019], thus the relevance of the nearly identical expression patterns of *PDR1* and *MDR1* between the SA and FB1 treatments may indicate that the proteins partially contributed to plant defense through the salicylic pathways.

Reversible phosphorylation plays a critical role in plant defense responses to pathogen attack [XING & LAROCHE, 2011]. Previously, components of mitogen-activated protein kinase (MAPK) cascades in wheat were shown to be part of the defense responses of wheat to leaf rust and *Fusarium* head blight infection [GAO & al. 2011, 2016]. The existence of multi-member families at each tier of the cascade is supported by recent genome-wide analysis [GOYAL & al. 2018; WANG & al. 2016]. To examine if MAP kinase pathways are involved in the activation of *PDR1* and *MDR1* by FB1 or SA, staurosporine and SB202190 were included the treatment. Our data seem to indicate an involvement of phosphorylation, but it may not be mediated through a p38 class of MAP kinase.

In silico analysis aided by Genevestigator suggests that with no biotic stresses both *MDR1* and *PDR1* had expression levels in all analyzed parts of a wheat plant. The expression strength is equitably distributed across different organs of the plant as no part of the plant was statistically different from the other (Figure 3). It should be noted that many ABCG/PDR-type ABC transporters are induced by biotic stresses. Among these are AtABCG40/AtPDR12 [CAMPBELL & al. 2003], AtABCG16 [JI & al. 2014], and potato StPDR1 to StPDR4 [RUOCCO & al. 2011]. A significant challenge is the fact that we still have not identified the substrate transported by Lr34. While our data further support the potential function of *MDR1* and *PDR1* in wheat defense responses, one of the main attempts should be the identification of the exact substrates of these transporters.

Notes on contributors

Kipkios TUBEI is a MSc student supported by a research grant from Bayer Crop Science Inc. Lucas CHURCH is an undergraduate student.

Tim XING is an associate professor and a plant molecular biologist with a special interest in cell signaling and plant-microbe interactions. He teaches plant physiology, molecular plant development, and cell signaling.

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